Fine-needle aspiration cytology of granulocytic sarcoma: A cytomorphologic analysis

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Abstract
Introduction: Granulocytic sarcoma is a rare extramedullary tumor comprising of immature myeloid cells in an extramedullary site which is commonly found with myeloid leukemia (acute or chronic) or occasionally with chronic myeloproliferative disorders or myelodysplastic syndrome. GS may also be the initial manifestation of leukemia. Because of the diverse clinical presentation, differentiation of Granulocytic sarcoma (GS) from malignant lymphomas (Non Hodgkin's lymphoma or Hodgkin's lymphoma), extramedullary hematopoiesis, poorly differentiated carcinoma and infections is very important for cytopathologist.

Materials and Methods: A retrospective study of total 16 cases of GS diagnosed by FNAC (14 FNAs and 2 pleural effusion fluid specimen) was done. Cytology smears were examined for cytromorphology in conjunction with clinical details. Depending upon the population of the cells present on the smears, GS was categorized as blastic, immature or mature GS.

Results: The age of patients ranged from 8 years to 50 years with male dominance. (10 males and 6 females). The most commonest site of aspiration was subcutaneous or soft tissue (9 cases) followed by lymphnodes (4 cases) and breast (1 case). 2 cases of GS involving pleural fluid were also included. A detailed history, clinical examination and relevant investigations of patients showed that GS was secondary to acute myeloid leukemia (AML) in 6 patients, was secondary to chronic myeloid leukemia (CML) in 3 patients, and was secondary to juvenile myelomonocytic leukemia (JMNL) in one patients. 6 patients were presented with concurrent myeloproliferative disorder [AML in 4 patients and CML-CP in 2 patients]. Depending upon the population of the cells present on the smears, GS was categorized as blastic GS (7 aspirates), immature GS (3 aspirates) and mature GS (6 aspirates).

Conclusion: FNAC plays important role in diagnosis of GS .GS should be included in the diagnosis of undifferentiated neoplasm of the soft tissue or lymph nodes, even in patients with no clinical suspicion of leukemia .Detailed patients clinical history along with careful morphologic evaluation for granulocytic cell identification should helps to reach a definitive diagnosis.

Keywords: Cytomorphology, Fine Needle Aspiration Cytology, Granulocytic Sarcoma.

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Introduction

Granulocytic Sarcoma (GS) or chloroma is localised tumor mass of extramedullary sites that is composed of immature cells of the granulocytic series.1 Davey et al2 introduced the term “Extramedullary myeloid cell tumor” to include both types of immature myeloid cell lesion that do not form destructive tumor masses as well as true GS.

GS usually associated with acute myeloid leukemia (AML), but can be found with chronic myeloid leukemia (CML) or other myeloproliferative disorders. It can be seen before, concurrent with or after the onset of myelogenous leukemia or other myeloproliferative disorders. GS is a tumorous manifestation of myelogenous leukemia and it represents the relapse in previously treated AML and development of blast crisis in chronic myeloid leukemia (CML) or other myeloproliferative disorders. Surprisingly, a proportion of patients never develop bone marrow disease.3 GS rarely is noted preceding the onset of leukemia.4

GS can be seen in many parts of the body but commonest sites of involvement are the lymph nodes, bone, soft tissue and skin. It also may present as a serous effusion.5,6

It is easy to establish the diagnosis of GS in patients with known hematologic disorders but in patients with no clinical suspicion of leukemia or in a previously healthy person, the differential diagnosis includes malignant lymphoma (Non Hodgkin's Lymphoma and Hodgkin's Lymphoma), infections, poorly differentiated carcinoma and other non lymphoid small round cell tumor.7
Fine Needle Aspiration Cytology (FNAC) is better and easy technique to establish the etiology of tumorous masses with accuracy, without resorting to open biopsies.

This study highlights the role of FNAC in diagnosis of GS, cytomorphological feature of GS as well as potential diagnostic pitfalls.

Material and Methods

A retrospective study of 16 cases of GS diagnosed by FNAC was done at Gujarat Cancer and Research Institute, Ahmedabad from January 2009 to December 2013. Two cases of GS diagnosed in the pleural fluid specimens were also identified.

Relevant clinical history, findings from peripheral blood and marrow, cytogenetic studies and results of flow cytometry analysis were obtained.

In all cases immunophenotyping was done at time of primary diagnosis.

FNAC was done by cytopathologist at cytology department using standard technique. Three to five passes from different areas of lesion were obtained. Aspirated material was smeared on to glass slides, immediately fixed in 100% methanol, and stained with modified Papanicolaou stain and one smear kept air dried for May Grunwald-Giemsa (MGG) staining. The cytology smears were examined by cytopathologist. Smears were reviewed for the morphology of the individual cells and to determine the proportion of blasts, immature myeloid precursor and granulocytic cells to categorized GS into three types: Blastic GS (with a predominance of blasts), Immature GS (with predominance of blasts and immature myeloid precursors) and Mature GS (with a full spectrum of granulocytic cells from blasts to granulocytes).

Results

Between January 2009 to December 2013, total 16 cases (14 FNAs and 2 Pleural effusion specimens) were identified. The age range was 8 to 50 years with male predominance. Patients characteristics are summarized in Table -1. Aspirated lesions vary in size from 1-5 cm with Mean diameter of 2 cm. The commonest sites of aspiration were subcutaneous and soft tissues (9 aspirates-scalp, face, eyelid, chest wall, forearm, gluteal region and perianal regions) followed by 4 lymph nodes (4 aspirates), breast (1 aspirates) and 2 cases of pleural effusion fluid also showed GS.

6 patients were found to have a concurrent myeloproliferative disorder comprising of AML (4 cases) and CML-CP (2 Cases). In 3 out of the 4 patient with concurrent AML, extramedullary relapse was confirmed by Born marrow examination while in 1 patient GS was the only manifestation of relapse without leukemic blood picture. 10 patients were known case of myeloproliferative disorders at the time they developed GS. These included 6 cases of AML, 3 case of CML-CP and 1 case of JMML. The interval varied from 1 month to 2 years from the initial diagnosis to manifestation of GS.

In 4 out of the 6 patients with known case of AML, relapse was confirmed by Born marrow examination. In the remaining 2 patients of AML, GS was the only presenting clinical manifestation of relapse without blood or Born marrow involvement by AML. In all the 5 patients of CML, extra medullary blast crisis appeared simultaneously with blast crisis in the blood or marrow. While in patient of JMML, GS was the only sign of blast crisis without blood and Born marrow involvement.

Cytomorphological features:

In the present study, depending on the cytomorphology we found blastic GS in 7 aspirates, immature GS in 3 aspirates and mature GS in 6 aspirates. The smears from blastic GS revealed a cellular aspirate showing monotonous population of dyscohesive cells-the feature characteristic of hematolymphoid tumors. The blast were medium to large in size (usually 2.5–4 times larger than a small mature lymphocytes) showing high N:C ratio, monomorphic round to oval regular nucleus with fine granular or dispersed chromatin, inconspicuous or small prominent nucleoli having scanty to moderate amount of pale blue to gray blue cytoplasm without cytoplasmic granules (Fig-1). Smears from immature GS revealed mainly large cells (blast or immature granulocytic precursors) with eccentrically placed nucleus showing irregular nuclear contour/nuclear indentation or lobation (depending upon the stage of maturation) with prominent nucleoli and scant to moderate amount of dull grey to eosinophilic grey cytoplasm showing variable cytoplasmic granules [Fig-2]. While aspirates from mature GS [Fig-3] showed mainly polymorphous population of cells comprising of admixture of mainly immature myeloid precursors (myelocytes, metamyelocytes and band forms), mature granulocytes with rare blasts.
Table 1: Clinical details and Cytological diagnosis of Granulocytic Sarcoma

<table>
<thead>
<tr>
<th>No</th>
<th>Pt Age</th>
<th>Gender</th>
<th>GS manifestation</th>
<th>Bone marrow</th>
<th>FNA site</th>
<th>GS</th>
<th>Specimens type and diagnosis Source</th>
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<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Male</td>
<td>Primary (AML)</td>
<td>Scalp</td>
<td></td>
<td></td>
<td>Mature</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Female</td>
<td>Secondary (JMML)</td>
<td>Face</td>
<td></td>
<td></td>
<td>Mature</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>Female</td>
<td>Secondary (AML)</td>
<td>Perianal</td>
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<td></td>
<td>Mature</td>
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<tr>
<td>4</td>
<td>27</td>
<td>Male</td>
<td>Secondary (AML)</td>
<td>Breast</td>
<td></td>
<td></td>
<td>Blastic</td>
</tr>
<tr>
<td>5</td>
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<td>Male</td>
<td>Primary (AML)</td>
<td>+ Eyelid</td>
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<td></td>
<td>Blastic</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>Female</td>
<td>Primary CML-CP</td>
<td>+ Forearm</td>
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<td>Immature</td>
</tr>
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<td>7</td>
<td>50</td>
<td>Male</td>
<td>Primary CML-CP</td>
<td>+ Chest</td>
<td></td>
<td></td>
<td>Mature</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>Male</td>
<td>Secondary (AML)</td>
<td>+ Leg</td>
<td></td>
<td></td>
<td>Mature</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>Male</td>
<td>Primary (AML)</td>
<td>+ Face</td>
<td></td>
<td></td>
<td>Blastic</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>Female</td>
<td>Primary (AML)</td>
<td>+ Pleural Fluid</td>
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<td></td>
<td>Blastic</td>
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<tr>
<td>11</td>
<td>38</td>
<td>Female</td>
<td>Secondary (AML)</td>
<td>+ Pleural Fluid</td>
<td></td>
<td></td>
<td>Blastic</td>
</tr>
<tr>
<td>12</td>
<td>36</td>
<td>Male</td>
<td>Secondary (AML)</td>
<td>+ Inguinal LN</td>
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<td></td>
<td>Blastic</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>Male</td>
<td>Secondary (CML-CP)</td>
<td>+ Cervical LN</td>
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<td></td>
<td>Blastic</td>
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<tr>
<td>14</td>
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<td>Secondary (CML)</td>
<td>+ Lymph node</td>
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<tr>
<td>15</td>
<td>26</td>
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</tr>
<tr>
<td>16</td>
<td>40</td>
<td>Female</td>
<td>Secondary (CML-CP)</td>
<td>+ Gluteal region</td>
<td></td>
<td></td>
<td>Mature</td>
</tr>
</tbody>
</table>

FNA: Fine needle Aspiration; GS: Granulocytic Sarcoma; AML: Acute Myelogenous Leukemia; JMML: Juvenile Myelomonocytic Leukemia; CML-CP: Chronic Myeloid Leukemia-Chronic Phase; CML: Chronic Myeloid Leukemia.

Pt Age Gender GS manifestation Bone marrow FNA site/ GS
No (Primary Vs Secondary) Involvement Specimens type and diagnosis Source

Fig. 1: Pleural Fluid Specimen showing Blastic Granulocytic Sarcoma. The patient had history of AML –M4 the blast demonstrate convoluted nuclei with single prominent nucleoli, scanty to moderate with cytoplasm along with normal mesothelial cells. (Papanicolaou stain, A X 400, B X 1000)
Fig. 2: Immature Granulocytic Sarcoma. Smears show mainly large cells with eccentrically placed vesicular nucleus with round or irregular nuclear outline, prominent nucleoli and scant to moderate dull grey cytoplasm mimicking Non-Hodgkin's Lymphoma. (Papanicolaou stain, X 1000)

Fig. 3: Aspirate from Face showing Mature Type Granulocytic Sarcoma. Smear shows mainly granulocytic cells in the different stage of maturation (myelocytes, metamyelocytes and band forms) with cytoplasmic granules -features consistent with myeloid differentiation. (Papanicolaou stain, X 1000)

Discussion

The present study highlights the clinical and cytomorphological spectrum of GS. GS is still frequently (up to 75%) erroneously diagnosed as malignant lymphoma attest to the difficulty of recognizing these lesions. GS is most commonly associated with AML but it may be found with chronic myeloproliferative diseases and myelodysplastic syndrome. It is interesting that this tumor may be the only primary manifestation of leukemia without appearance of the disease in blood or marrow.8,9

As previously noted by Neiman et al10, the prognostic significance of GS depends entirely on the clinical context in which it occurs. (often requiring ancillary, clinical and pathological studies for accurate assessment). In patients with concurrently diagnosed AML, the presence of GS has not been shown to have clinical or prognostic significance. On the other hand, GS in a patient previously treated for AML, represents relapse of disease. In our study, 10 out of the 16 patients were known case of AML, 4 patients found to have concurrent AML while 6 patients were previously treated for AML at the time, they developed GS. The development of GS in patients with known myeloproliferative disorders is associated with poor prognosis with median survival as short as 3 to 6 months. In our study, majority of cases whether the underlying disease was CML or another myeloproliferative disorder, tumor manifest as blast crisis. It is interesting that GS may develop at time in the evolution of a patient with myeloproliferative disorder in which the disease appears in all other clinical and hematologic respects to be stable. It is obvious that the clinical significance of diagnosis of GS in such patients is great as it strongly suggest that blast crisis is impending.10

Not all patients with GS present with concurrent associated hematologic disease, making the assessment difficult at initial presentation. With follow up of 3.5-16 years, Meis et al8 found that acute leukemia did not develop in 4 of 16 patients, who primarily manifested with GS Confounding the varied clinical presentation, no body site is spared as GS have been reported in the wide variety of anatomic location (8,10,11,12)-lymphnodes being consistently the most common single site in above studies. In our study, extranodal involvement were common. This propensity to involve extranodal sites suggests GS should be kept in mind in the differential diagnosis of undifferentiated hematological malignancy comprising of intermediate or large sized cell in unusual location outside the lymphoid organs.

In our study, cytomorphologically we have categorized GS as blastic, immature and mature type depending upon the population of granulocytic series cells in aspirated material. A study conducted in the histology specimens, by Neiman et al10 also divided GS in to three types. (1) well differentiated GS -if many eosinophilic myelocytes were present in any section of a given case; (2) blastic GS -when there was no morphologic evidence of granulocytic differentiation and (3) poorly differentiated GS -when only occasional eosinophilic as myelocytes present in the histology section. Similarly, Traweek et al13 also divided GS into three groups depending upon the degree of granulocytic differentiation as well differentiated, poorly differentiated and blastic type. When GS occurs
in patients without previous history of any hematolymphoid neoplasm and the tumor show little or no evidence of myeloid differentiation, the diagnosis of GS can be very difficult and sometimes impossible by using routine technique. In such condition immune peroxidase studies, first using polyclonal antisera followed by monoclonal antibodies seems to play important role in the diagnosis of GS and probably represent the best and most accessible method for ancillary study of these lesions.

The differential diagnosis of GS may vary depending upon the stage of granulocytic cell differentiation (mature granulocytes, granulocytic precursors and blasts) present on the aspirates. Immature or mature type of GS, easily confused with Lymphoma (Hodgkin’s Disease or T-cell lymphomas), extramedullary hematopoiesis or inflammatory conditions due to presence of polymorphous cell population in the aspirates while blastic or immature GS should be differentiated from Non Hodgkin’s lymphoma (large cell type/lymphoblastic lymphoma), carcinoma or even melanoma, without considering the site of aspiration (lymph node or extra nodal region). The Cytopathologist must be aware of GS and should carefully examine a lesion that resembles a Non Hodgkin’s Lymphoma or even undifferentiated malignant tumor. If the tumor resemble lymphoma but the nuclear characteristics does not fit any of the known B-cell or T-cell lymphoma categories, pathologist must be alerted to the diagnosis of GS. The nucleus shows characteristic multilobed appearance in GS. As described by Meis et al., the nuclear chromatin is extremely fine or dusty, and there are 1 or 2 small basophilic nucleoli. In large cell lymphoma, the nuclei are much less lobated than in GS and the chromatin is more clumped and coarse while the nucleoli are larger and more prominent. The cytoplasm is more prominent in GS. There is absence of eosinophils and metamyelocytes in Non Hodgkin’s lymphoma and in any carcinomas while presence of eosinophils and Reed-Sternberg cells can differentiate Hodgkin’s disease from GS. Combined use of ancillary techniques like immunophenotyping or immunohistochemistry along with clinical history may help to reach the diagnosis of GS over Lymphoma, Carcinoma and Melanoma in difficult cases. Another important differential diagnosis of immature or mature GS is Extramedullary hematopoiesis which is more frequently associated with hematologic diseases like leukemia, myelofibrosis and myelopathic anemia in elderly and can present in any anatomic site in of body including the liver, spleen breast, lymphnodes, kidneys, thyroid, pancreas, endometrium and mediastinum or can present in serous effusion. Smears from Extramedullary haemopoiesis usually shows erythroid, myeloid and megakaryocytic cells with predominance of one lineage in the aspirated material. The mature or immature mononuclear megakaryocytes can mimick malignant neoplastic cells.

When GS shows predominantly mature granulocytes, the closest and important differential diagnosis is infectious process as it affects the treatment modality. Identification of whole spectrum of granulocytic precursors (Myeloblast, promyelocyte or myelocytes) confirm a diagnosis of GS while presence of inflammatory infiltrate, necrotic material along with use of special stains (ZN stain, PAS stain) to identify the microorganisms leads to diagnosis of an infectious process over GS.

**Conclusion**

GS is an uncommon tumor with difficult clinical and morphologic recognition. The Present Study highlights that well differentiated tumors are frequently misdiagnosed as malignant lymphoma when they present as the primary manifestation without any history of antecedent or concurrent leukemia. It has to be kept in the differential diagnosis of undifferentiated neoplasm arising in any anatomic site. A thorough study of the cytomorphology of the tumor cells for evidence of granulocytic differentiation and high index of suspicion when confronted with a less differentiated neoplasm are required to avoid this important diagnostic error.

Thus, morphological examination of the smears gained by FNAC was sufficient in most of our cases and GS could be diagnosed based on cytology findings.

**References**